NATIONAL CHILDREN'S STUDY
ENVIRONMENT AND ADULT REPRODUCTIVE HEALTH CORE HYPOTHESIS

I. Proposed hypothesis

Environmental exposures during pre- and post-natal development effect adult reproductive health and function.

II. Workgroup

Fertility & Early Pregnancy (Possible collaboration with Growth; Exposure to Chemical Agents)

III. Contact persons

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IV. Public Health Significance

The last ten years or so have produced a body of evidence indicating that many environmental xenobiotics are having hormone-like effects on wildlife. Endocrine active compounds (EACs), which also are referred to as Endocrine Disrupting Chemicals, EDCs, have been defined as exogenous agents that interfere with the production, release, transport, metabolism, binding, action, or elimination of the natural hormones in the body responsible for the maintenance of homeostasis and the regulation of developmental processes (Kavlock et al., 1996). The potential threat of such agents to human reproductive health has been heralded by a number of well-documented cases in which wildlife, exposed to specific, known pollutants, or mixtures of pollutants, has suffered measurable adverse effects on reproductive health. Some of the best known cases include changes in the reproductive organs of mature female neogastropods exposed to tributyltin (Gibbs, 1996; Gibbs & Bryan, 1996), impaired testicular development and production of vitellogenin in male fish exposed to estrogenic alkylphenols and human estrogens in sewage effluent (Sumpter, 1995), and abnormal development of reproductive organs in alligators exposed to the organochlorine pesticide, dicofol and DDT or its metabolites (Guillette et al., 1994,1996). Similar adverse reproductive effects have also been demonstrated in laboratory species (Cooper and Kavlock, 1997). An increasing amount of circumstantial human evidence, including a purported reduction in semen quality, an increase in cryptorchidism and hypospadias, and an increase in testicular, prostate and female breast cancer, has contributed to speculation that exposure to environmental EACs may be linked to a number of adverse reproductive health effects in humans.

Numerous adverse reproductive outcomes have been positively associated with adult exposures to environmental toxicants (such as pesticides), including sperm aneuploidy / hyperploidy / polyploidy (Recio et al., 2001), disruption of ovarian cycles (Cooper et al., 1996) and an increased rate of spontaneous abortions (Arbuckle et al., 2001). Until recently, the study of reproductive effects has been focused primarily on adult individuals. However, in view of the

biopersistence of many environmental xenobiotics and the recognized threat from cumulative and aggregate exposures, children's health has become an area of increasing concern as the possibility of an adverse impact of such agents on ensuing reproductive development and health cannot be discounted. Furthermore, since many pesticides (one of the largest source of environmental EACs) are biopersistent, it is possible that exposure to even moderate levels of such chemicals during growth and development will result in their accumulation in the body, leading to adverse reproductive effects manifesting at a later juncture.

There is unequivocal evidence that environmental factors can play a significant role in altering normal growth and development, and it is widely recognized that the *in utero* stage of development is particularly susceptible to such exposures. Indeed, many and varied adverse outcomes have been described in children of mothers who have knowingly or unknowingly used, or been exposed to, "recreational" drugs (alcohol, tobacco), drugs of abuse (e.g. heroine, cocaine, cannabis), pharmaceutical drugs, and environmental toxicants. The effects of such exposures on reproductive function are not well characterized in humans however, since the time between exposure and measurable outcome (reproductive health) is long, and suitable cohorts (usually derived through large scale accidental contamination of food sources or incorrect drug prescription) are rarely available. The DES exposure story is exceptional in this case, and proved that *in utero* exposure to an EAC can impact adult reproductive health (women were found to have many fertility problems (Goldberg & Falcone, 1999), whilst men seemed unaffected (Wilcox et al., 1995). Many animal studies have also shown that *in utero* exposures can have adverse effects on adult reproductive health. For example, *in utero*/lactational exposure to dioxin causes abnormal mammary gland differentiation in female rats (Lewis et al., 2001), and in males both reduces the weight of the urogenital complex (Ohsako et al., 2001) and decreases sperm numbers (Sommer et al., 1996). Other chemicals having adverse effects on reproductive health following gestational/lactational exposures include PCBs, phthalates and organochlorine and organophosphate pesticides.

There is equal concern that postnatal exposures are the source or contributing factor to some adult abnormalities. In animal models, postnatal exposure to dioxin or dioxin-like compounds has been associated with abnormal spermatogenesis and abnormal testicular morphology and size in males, and with genital dysmorphogenesis (Flaws et al., 1997), impaired mammary gland differentiation (Lewis et al., 2001), reduced fertility and endometriosis in females.
Nearly all human studies to date on the impact of exposure to environmental toxicants on reproductive health has been carried out in adults, and reduced fertility/fecundity as a result of *in utero*, lactational or juvenile exposures has not been well evaluated. For the most part, such studies have been limited to pharmacokinetic evaluations and concentrations of the toxicants in blood, serum and urine.

The actual public health significance of the impact of gestational, lactational and juvenile exposure to toxicants on adult reproductive health cannot be fully assessed at this time as the actual contribution of such exposures to reproductive health is uncertain. However, there is significant environmental and laboratory evidence suggesting that gestational and developmental exposure to environmental toxicants, particularly EACs, does indeed impact adult reproductive health. With respect to quality of life, abnormal reproductive organs are clearly of psychological

concern to those who have them. Quality of life is further impacted for those who wish to have children but cannot. Inability to conceive is clearly a source of emotional anguish to infertile couples, and the cost of fertility treatment has escalated significantly as assisted reproduction technologies (ARTs) have become more widely accepted and available.

V. Justification for a large, longitudinal study

A large longitudinal approach allows the examination of a wide variety of exposures and measurement of multiple indicators of reproductive health over the time frames of potential importance in the sexual maturation process. This is in keeping with the highly interrelated processes underlying successful human reproduction and development.

VI. Scientific Merit

(1) With regard to exposure to EACs, the level of fetal exposure is uncertain. Other methodological factors requiring clarification include: (a) the vast number of chemicals described as EACs, (b) the ability of chemicals to bioaccumulate, (c) the metabolism of body lipids during pregnancy and lactation, releasing the mothers lifetime EAC accumulation into circulation, (d) the poorly understood kinetics of EAC transfer across the placenta. (e) The effect of mixtures of EACs. Thus, the level of fetal exposure can only be crudely estimated at present and more knowledge is required to overcome these limitations.

(2) There is a need to be able to better extrapolate the results of animal studies to humans. Use of physiologically based pharmacokinetic [PBPK] and biologically based dose-response [BBDR]) models are relevant for understanding changes during pregnancy and lactation. Collection of data as proposed above will allow for the development of such models.

(3) There have been a number of recent studies confirming the presence of known toxicants in amniotic fluid (Foster et al., 2000), fetal blood and maternal milk (Hwang et al., 2001), at least one of which have found levels of toxicants above thresholds at which developmental impairment is known to occur (Muckle et al., 1998). However, there is limited information on the impact of *in utero*/lactational exposure to toxicants on the subsequent growth and development of children. Neurological (Grandjean et al., 2001), behavioral (Kimbraugh et al., 2001), immunological (Dewailly et al., 2000) and other tests can be conducted at an early age, and in this way certain exposures have been correlated with developmental effects in children (e.g. *in utero* PCB exposure and middle ear diseases, Chao et al., 1997). However, reproductive studies of exposed cohorts have been very limited. One exception is a study in Taiwan of boys accidently exposed *in utero* to PCB-contaminated rice oil; the boys developed smaller penises as they matured compared to the controls (Holloway, 1994). It is interesting that alligators exposed to pesticides in Florida have undersized penises (Guillette et al., 1996).

(4) The lack of human data means that animal studies on laboratory and wildlife species have served as the main source of information and hypotheses. Interestingly, Cooke et al. (1996) found that in rats, PCBs, despite inhibitory effects on adult reproductive organs, can paradoxically stimulate increases in adult testis weight and daily sperm production when

administered neonatally. These data emphasize the pleiotropic nature of PCB effects and the susceptibility of the developing reproductive system to environmental factors, and indicate that other chemicals could have similar effects.

Studies carried out in support of this hypothesis will help determine the degree to which adult reproductive health is actually impacted by *in utero*, lactational and juvenile exposures, and the range of exposures that can induce the observed effects. It will provide normative data on the contribution of genetic makeup and variation in susceptibility within regions and amongst racial/ethic groups, and thus permit identification of at-risk and susceptible populations and critical windows of exposure.

Such normative data on developmental windows will improve risk assessment by identifying the most sensitive window(s) for evaluation of dose-response relationships and exposure, evaluation of biological plausibility of research findings in humans, and comparison of data across species. In public health and risk management, information on critical windows may help identify especially susceptible subgroups for specific interventions.

VII: Potential for innovative research

(1) There are a huge number of xenobiotic chemicals found in the environment, many of which are known or suspected to disrupt reproductive development. High throughput methods to detect the presence of a broad spectrum of xenobiotics and/or their metabolites in human tissue samples will be needed for routine biomonitoring of human populations, particularly those known to be in locales or occupations with elevated exposures to known toxicants.

(2) The identification of biomarkers of exposure and effects of such reproductive toxicants could be one of the most significant areas of reproductive health research to emerge over the next 20 years. The power of a large study such as the National Children's Study will facilitate this aim with samples from both the parental and next generations

(3) Sperm viability is particularly important in assessing the causes of individual infecundity and related fertility impairments, as well as being important to those in the ART field selling or using donor semen. Therefore it is crucial to develop and use accurate diagnostic tests to determine the fertility of males and of individual semen samples. Such diagnostic tests should be economically practical and provide consistent results. Sperm biomarkers of fertility offer one such approach to assessing the viability of sperm. For example, SP22 is a sperm-expressed protein that has been highly correlated with, and predictive of, the fertilizing ability of cauda epididymal sperm. (Klinefelter et al., 1997). Multiple other biomarkers of sperm fertility have been suggested, including Hyaluronan binding protein 1, fibronectin, infertility-associated sperm protein and progesterone receptor. However, all of these will need further investigation into their sensitivity, efficiency and practical utility.

(4) It has recently been shown that RNA can be extracted from human sperm (Miller et al, 1999). Although there is controversy over the functional significance of such RNA, it is possible that the genetic information that can be derived from the RNA e.g. through gene expression profiling,

could be used to assess fertility and perhaps provide information on the genetic or environmental cause of infertile or subfertile samples.

(5) The ovulatory cycle is a key endpoint for assessing reproductive health. Kits for detection of ovulation and pregnancy are evolving, and will be able to generate high quality data key checkpoints in the cycle that will be useful in determining its normality.

VIII. Feasibility

Critical period for exposure and outcomes: Limited data are available, but need to correlate developmental exposures with adult reproductive health. Therefore probably a continuous sampling process within growth and development, but with particular emphasis being placed on the gestational, perinatal and pubertal developmental stages, which are seen as being particularly susceptible to disruption by environmental toxicants.

Sampling needs: Certain communities may be more exposed – those who eat more fish from sources known to be high in environmental contaminants such as methylmercury; those who are breast fed; those who live near (downriver, downwind) of known point sources of contamination; drug addicts.

Contact: To be determined by a proposed study. Possibly to include: Diary and biological (blood, hair, urine) samples from mother during pregnancy to assess internal exposure to toxicants through food or other routes. Milk samples to measure level of chemicals being passed on to breast-fed babies through the mother. During child puberty, periodic food or activity diaries to be kept by participants. Periodic biological (blood, hair, urine) samples required to test for the presence of EACs etc. Tracking of teenage pregnancy incidence and outcome. After age 18, assess semen in males and normal cycling in females. Physical exam at age 21 (exit exam).

Nature of measurement: As above – periodic clinical exams; biological samples such as urine, blood, hair and semen.

Burden on participant and family: Possibly medium at times (diaries). Otherwise minimal as most samples collected during routine checkups. Subjects may be required to come in periodically outside scheduled examinations. Counseling for discovery of adverse effects.

Ethical considerations: 1. Use of minimally invasive procedures preferable; 2. Privacy of study participants and confidentiality of data; 3. Obtaining informed consent may involve more than one generation; 4. Consequence of adverse findings; 5. Provision of medical care upon discovery of adverse findings; and 6. Payment of medical services for participants who belong to health organizations (standard v non-standard).

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